

CLAIMS

What is claimed is:

1. A nucleic acid segment comprising an isolated
gene encoding a lipoxxygenase, said lipoxxygenase containing
5 an iron ligand comprising a serine.
2. The nucleic acid segment of claim 1, wherein said
isolated gene encodes a polypeptide having an *in vivo*
molecular weight of about 76 KD when measured by SDS-PAGE.
3. The nucleic acid segment of claim 1, wherein the
10 encoded lipoxxygenase converts arachidonic acid exclusively
to 15S-hydroperoxyeicosatetraenoic acid or converts
arachidonic acid exclusively to 8S-
hydroperoxyeicosatetraenoic acid.
4. The nucleic acid segment of claim 1, wherein the
15 isolated gene encodes 15-Lox-2 or 8-Lox.
5. The nucleic acid segment of claim 1, further
defined as a DNA segment.
6. A recombinant host cell comprising the nucleic
acid segment of claim 1.
- 20 7. The nucleic acid segment of claim 4, wherein the
isolated gene encodes 15-Lox-2.
8. The nucleic acid segment of claim 4, wherein the
isolated gene encodes 8-Lox.

9. The nucleic acid segment of claim 7, wherein the isolated gene encodes 15-Lox-2 comprising the amino acid sequence of SEQ ID NO:2.

10. The nucleic acid segment of claim 9, further defined as comprising the 15-Lox-2-coding nucleic acid sequence of SEQ ID NO:1.

11. The nucleic acid segment of claim 8, wherein the isolated gene encodes 8-Lox comprising the amino acid sequence of SEQ ID NO:4.

12. The nucleic acid segment of claim 11, further defined as comprising 8-Lox-coding nucleic acid sequence of SEQ ID NO:3.

13. The nucleic acid segment of claim 5, wherein the isolated gene is positioned under the control of a promoter.

14. The nucleic acid segment of claim 13, further defined as a recombinant vector which comprises the isolated gene.

15. The nucleic acid segment of claim 14, wherein the vector is a recombinant expression vector.

16. The recombinant host cell of claim 6, wherein the host cell is a procaryotic cell.

17. The recombinant host cell of claim 6, wherein the host cell is a eucaryotic cell.

18. A nucleic acid segment which comprises at least a 10 nucleotide long contiguous stretch of the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:3.

19. The nucleic acid segment of claim 18, further
5 defined as comprising at least a 15 nucleotide long contiguous stretch of the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:3.

20. The nucleic acid segment of claim 19, further
10 defined as comprising at least a 20 nucleotide long contiguous stretch of the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:3.

21. The nucleic acid segment of claim 19, further defined as a nucleic acid fragment of up to 10,000 basepairs in length.

15 22. The nucleic acid segment of claim 20, further defined as comprising at least a 30 nucleotide long contiguous stretch of the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:3.

20 23. The nucleic acid segment of claim 22, further defined as comprising at least a 50 nucleotide long contiguous stretch of the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:3.

24. The nucleic acid segment of claim 23, further defined as comprising at least a 100 nucleotide long

contiguous stretch of the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:3.

25. The nucleic acid segment of claim 24, further defined as comprising at least a 1000 nucleotide long
5 contiguous stretch of the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:3.

26. The nucleic acid segment of claim 25, further defined as having the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:3.

10 27. The nucleic acid segment of claim 21, further defined as a nucleic acid fragment of up to 1,000 basepairs in length.

28. The nucleic acid segment of claim 27, further defined as a nucleic acid fragment of up to 500 basepairs
15 in length.

29. The nucleic acid segment of claim 28, further defined as a nucleic acid fragment of up to 50 basepairs in length.

30. A method of preparing a lipoxxygenase polypeptide,
20 comprising: transforming a cell with the nucleic acid of claim 1 to produce a lipoxxygenase under conditions suitable for the expression of said polypeptide.

31. A process of detecting in a sample an RNA that encodes the lipoxxygenase polypeptide encoded by the

nucleic acid of claim 1, said process comprising the steps of:

(a) contacting said sample under hybridizing conditions with the nucleic acid segment of claim 1 to form a duplex; and

(b) detecting the presence of said duplex.

32. An isolated and purified biologically active lipoxxygenase polypeptide capable of converting arachidonic acid exclusively to 15S-hydroperoxyeicosatetraenoic acid, said lipoxxygenase containing an iron ligand comprising a serine.

33. A polypeptide of claim 32, wherein said polypeptide has an *in vivo* molecular weight of about 76 KD when measured by SDS-PAGE.

34. A polypeptide of claim 32, further comprising an amino acid sequence W-L-L-A-K (SEQ ID NO:5) and an amino acid sequence G-Q-Y-D-W (SEQ ID NO:35), the amino acid sequence W-L-L-A-K (SEQ ID NO:5) positioned upstream from the amino acid sequence G-Q-Y-D-W (SEQ ID NO:35) along the polypeptide.

35. A polypeptide according to claim 32, wherein the polypeptide comprises a 15-Lox-2.

36. A polypeptide according to claim 35, wherein the 15-Lox-2 comprises the amino acid sequence of SEQ ID NO:2.

37. A polypeptide according to claim 32, modified to be in detectably labeled form.

38. An isolated and purified antibody capable of specifically binding to the polypeptide of claim 32.

5 39. The antibody of claim 38 which is a monoclonal antibody.

40. The antibody of claim 38 which is a polyclonal antibody.

10 41. A hybridoma cell line which produces the monoclonal antibody of claim 39.

42. An isolated and purified antibody capable of neutralizing the biological activity of the polypeptide of claim 32.

15 43. The antibody of claim 42 which is a monoclonal antibody.

44. The antibody of claim 42 which is a polyclonal antibody.

45. A hybridoma cell line which produces the monoclonal antibody of claim 43.

20 46. A process of producing an antibody immunoreactive with a lipoxxygenase polypeptide, the process comprising steps of

(a) transfecting a recombinant host cell with the a polynucleotide of claim 1, which encodes a lipoxxygenase polypeptide;

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- (b) culturing the host cell under conditions sufficient for expression of the polypeptide;
- (c) recovering the polypeptide; and
- (d) preparing the antibody to the polypeptide.

5 47. The process of claim 46, wherein the polypeptide comprises SEQ ID NO:2.

48. The process of claim 46, wherein the polynucleotide comprises SEQ ID NO:1 or comprises SEQ ID NO:3.

10 49. An antibody produced by the process of claim 46.

50. A process of detecting a lipoxxygenase polypeptide, the process comprising immunoreacting the polypeptide with an antibody prepared according the process of claim 46 to form an antibody-polypeptide
15 conjugate, and detecting the conjugate.

51. A process of detecting a messenger RNA transcript that encodes a lipoxxygenase polypeptide, the process comprising the steps of hybridizing the messenger RNA transcript with the polynucleotide of claim 1 to form
20 a duplex; and detecting the duplex.

52. A process of detecting a DNA molecule that encodes a lipoxxygenase polypeptide, the process comprising the steps of hybridizing DNA molecules with the polynucleotide of claim 1 to form a duplex; and detecting
25 the duplex.

53. A diagnostic assay kit for detecting the presence of a lipxygenase polypeptide in a biological sample, the kit comprising a first container containing a first antibody capable of immunoreacting with a lipxygenase polypeptide encoded by the polynucleotide of claim 1, wherein the first antibody is present in an amount sufficient to perform at least one assay.

54. An assay kit of claim 53, further comprising a second container containing a second antibody that immunoreacts with the first antibody.

55. An assay kit of claim 54, wherein the first antibody and the second antibody comprise monoclonal antibodies.

56. An assay kit of claim 55, wherein the first antibody is affixed to a solid support.

57. An assay kit of claim 55, wherein the first and second antibodies each comprise an indicator.

58. An assay kit of claim 57, wherein the indicator is a radioactive label or an enzyme.

59. A diagnostic assay kit for detecting the presence, in biological samples, of a lipxygenase polypeptide, the kit comprising a first container that contains a polynucleotide identical or complimentary to a segment of at least ten contiguous nucleotide bases of the polynucleotide of claim 1.

60. A diagnostic assay kit for detecting the presence, in a biological sample, of an antibody immunoreactive with a lipxygenase polypeptide, the kit comprising a first container containing a lipxygenase
5 polypeptide encoded by the polynucleotide of claim 1 that immunoreacts with the antibody, with the polypeptide present in an amount sufficient to perform at least one assay.

61. A screening assay for identifying a compound
10 that affects arachidonic acid metabolism in a cell, comprising the steps of:

- (a) establishing replicate test and control cultures of cells that express a lipxygenase polypeptide encoded by the polynucleotide of claim 1;
- 15 (b) administering a candidate compound to the cells in the test culture but not the control culture;
- (c) measuring hydroperoxyeicosatetraenoic acid levels in the test and the control cultures; and
- (d) determining that the candidate compound affects
20 arachidonic acid metabolism in a cell if the hydroperoxyeicosatetraenoic acid level measured for the test culture is less or greater than the hydroperoxyeicosatetraenoic acid level measured for the control culture.

62. An assay of claim 61, wherein the lipxygenase polypeptide comprises 15-Lox-2.

63. An assay of claim 61, wherein the lipxygenase polypeptide comprises 8-Lox.